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Antimalarial and antitubercular nostocarboline and eudistomin derivatives: Synthesis, in vitro and in vivo biological evaluation

Simone Bonazzi [a], Damien Barbaras [b], Luc Patiny [c], Rosario Scopelliti [c], Patricia Schneider [d], Stewart T. Cole [d], Marcel Kaiser [e], Reto Brun [e], Karl Gademann [a,*]

[a] Chemical Synthesis Laboratory (SB-ISIC-LSYNC), Swiss Federal Institute of Technology (EPFL), CH-1015 Lausanne, Switzerland
[b] Laboratorium für Organische Chemie, Swiss Federal Institute of Technology (ETH), CH-8093 Zürich, Switzerland
[c] Institute of Chemistry and Chemical Engineering (ISIC), Swiss Federal Institute of Technology (EPFL), CH-1015 Lausanne, Switzerland
[d] Global Health Institute, Swiss Federal Institute of Technology (EPFL), CH-1015 Lausanne, Switzerland
[e] Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute (STPH), Socinstrasse 57, CH-4002 Basel, Switzerland

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ABSTRACT

The synthesis of nine nostocarboline derivatives with substitutions of the 2-methyl group by alkyl, aryl and functionalized residues, 10 symmetrical bis cationic dimers linking 6-Cl-norharmane through the 2-position and fifteen derivatives of the marine alkaloids eudistomin N and O is reported. These compounds were evaluated in vitro against four parasites (*Trypanosoma brucei rhodesiense* STIB 900, *Trypanosoma cruzi* Tulahuen C2C4, *Leishmania donovani* MHOM-ET-67/L82 axenic amastigotes, and *Plasmodium falciparum* K1 strain), against *Mycobacterium tuberculosis* H37Rv, *Mycobacterium smegmatis* mc^2155 and *Corynebacterium glutamicum* ATCC13032, and cytotoxicity was determined against L6 rat myoblast cells. Nostocarboline and derivatives displayed potent and selective in vitro inhibition of *P. falciparum* with weak cytotoxicity. The dimers displayed submicromolar inhibition of *L. donovani* and *T. brucei*, and nanomolar activity against *P. falciparum*, albeit with pronounced cytotoxicity. One dimer showed a MIC_{99} value against *M. tuberculosis* of 2.5 μg/ml. The alkylated eudistomin N and O derivatives displayed activities down to 18 nM against *P. falciparum* for *N*-Me Eudistomin N. Four dimers, nostocarboline and three eudostomin derivatives were evaluated in an in vivo *Plasmodium berghei* mouse model. No significant activity was observed for the dimers, but a 50% reduction in parasitaemia was observed at 4 × 50 mg/kg ip for nostocarboline.

1. Introduction

Infectious diseases such as malaria, tuberculosis, leishmaniasis or sleeping sickness are considered major public health challenges in developing countries.[1] For malaria alone, over 1 million deaths are estimated per year, and the number of clinical cases is thought to be 100 times higher.[2] Parasite chemotherapy remains the therapeutic option of choice, given the absence of a vaccine and the difficulties associated with systematic vector control.[3] However, the emergence of malaria cases in non-endemic areas combined with the rise of multidrug-resistant parasites calls for the development of novel drugs against this disease.[3] A similar situation is observed related to tuberculosis, where a diminished efficacy of currently used drugs combined with the emergence of resistant strains also emphasizes the need to discover novel leads.[4]

Natural products have been successfully employed for decades in the search for novel biologically active compounds, and to date, roughly 50% of all anti-infective drugs are natural products or derivatives thereof.[5] Against malaria, quinine and its derivatives are still used today, and combination therapies relying on artemisinin are currently the treatment of choice.[3,5] The search for novel lead structures from natural sources such as plants, bacteria or marine organisms is thus warranted.[6]

Cyanobacteria are an interesting source of novel metabolites[7] and a large diversity of compounds such as peptides, peptolides, polyketides, terpenes and alkaloids is documented in the literature.[7,8] These prokaryotic phototrophs face ecological pressure from both grazers and competing organisms, which they address by producing bioactive secondary metabolites. Several compounds display allelochemical properties in inhibiting the growth of competing phototrophs.[9] Such phytotoxic or algicidal compounds have been shown to display activity against *Plasmodium*,[10] as this parasite contains an organelle of algal (cyanobacterial) origin, the apicoplast.[11] Based on this experimental evidence, we have been employing a chemical ecology strategy for the search of novel antiplasmodial compounds based on their allelochemical properties in cyanobacteria. We have characterized the heterocyclic macrocyclic

* Corresponding author. Tel.: +41 21 693 93 15; fax: +41 21 693 97 00.
E-mail address: karl.gademann@epfl.ch (K. Gademann).

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peptide aerucyclamide B,[8b] which displays submicromolar activity against *Plasmodium falciparum* K1 and only weak cytotoxicity.[8c] These results are in line with other studies of similar peptides such as venturamides A and B.[12] Interestingly, this class of peptides has been shown earlier to possess allelochemical properties towards other phototrophic organisms,[13] thus validating our chemical ecology approach. Another literature example is the antimalarial agent calothrixin A,[14a] which also displays allelochemical properties.[14b] Several other antiplasmodial natural products have been described from cyanobacteria that might also involve allelochemical properties.[14c–e]

Another compound that underlines the value of this strategy is nostocarboline (**1**) or *N*-methyl-6-chloro-carbolinium, which was isolated from the cyanobacterium *Nostoc* 78-12A.[15] Nostocarboline was shown to inhibit hydrolytic enzymes such as acetyl and butyryl cholinesterase, and trypsin,[15,16] while only displaying very weak toxicity against the sensitive freshwater crustacean *Thamnocephalus platyurus*.[16] We have shown earlier that nostocarboline (**1**) displays allelochemical properties in inhibiting the growth of both eukaryotic and prokaryotic photosynthetic organisms, while being inactive (MIC >93 μM) against non-photosynthetic pathogens.[17a] This inhibition of the photosystem was confirmed by chlorophyll-*a* fluorescence imaging, which demonstrated that inhibition of photosynthesis preceded cell death.[17b] Taking advantage of this selective action against phototrophs,[17] we tested nostocarboline and ten dimeric derivatives in vitro against a series of protozoan parasites and found selective and potent in vitro activity.[18] In this article, we report in full detail the synthesis and the biological in vitro evaluation of additional nostocarboline derivatives, demonstrate in vivo activity of nostocarboline (**1**) in the *Plasmodium berghei* mouse model, evaluate mechanistic details and report additional activity of the nostocarboline dimers against *Mycobacterium tuberculosis*. In addition, we report on the synthesis of N-alkylated derivatives of eudistomin N[19] (**2**) and eudistomin O[19] (**34**) and also their biological in vitro and in vivo evaluation.

2. Results and discussion

Nostocarboline, which was originally prepared as the hydroiodide salt **1**,[15] constitutes an anhydronium base, which can be represented by two different resonance structures **3a** and **3b**. We have prepared nostocarboline anhydronium base by treating nostocarboline (**1**) with aqueous NaOH, and a change in color and fluorescence became visible: whereas nostocarboline (**1**) is yellow-brown and emits blue fluorescence, the corresponding base **3a** is yellow and emits a strong yellow fluorescence. We were able to obtain crystals of the anhydronium base that were subjected to X-ray crystallographic analysis (Fig. 1). As the anhydronium base is relatively weak (pK_a around 10), it is reasonable to assume that at physiological pH a minor fraction of **1** is present in the deprotonated state **3**, which could be beneficial in crossing cellular membranes. Moreover, nostocarboline (**1**) features a Cl group—unusual for a freshwater metabolite, which certainly adds lipophilicity to the compound. We think that these chemical features of the anhydronium base in combination with the Cl substituent might be crucial for antimalarial activity.

The synthesis of the new nostocarboline derivatives follows a short and straightforward route, and relies on inexpensive starting materials such as tryptophan that can be converted to norharman in two steps (Scheme 1).[15] We think that these parameters are an important requirement for successful drugs against tropical infectious diseases. The synthesis of analogs of nostocarboline starts from 6-Cl-norharmane (**4**), which itself is accessible via chlorination of norharmane.[15] Alkylation of carboline **4** with a series of electrophiles directly afforded the nostocarboline derivatives **5–12**. The dimers **13–22** are obtained by treating 6-Cl-norharmane with a series of different symmetrical dihalogenated linkers. We were able to employ alkyl, aryl, alkenyl and alkynyl linkers of varying length and the corresponding products were obtained in good to excellent yields.

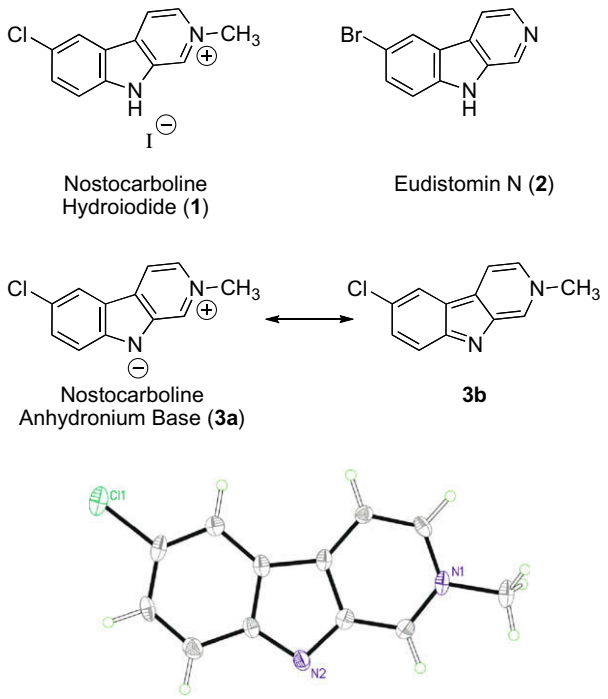
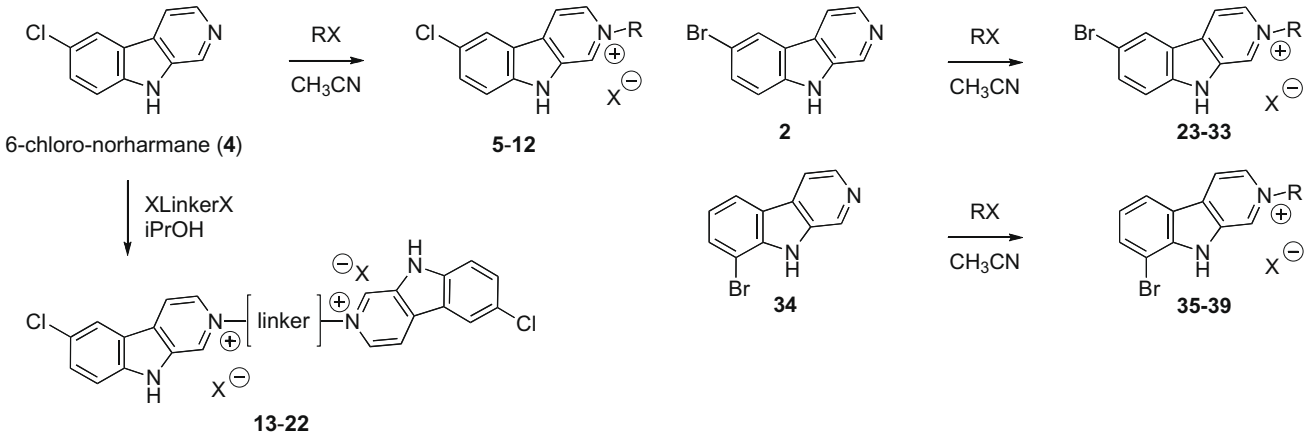


Figure 1. Formulae of nostocarboline hydroiodide (**1**), eudistomin N (**2**), resonance structures **3a** and **3b** of nostocarboline anhydronium base, and X-ray crystal structure of **3**.

The preparation of the N-alkylated eudistomin derivatives followed a similar short and straightforward synthesis. Norharmane was brominated using NBS to afford eudistomin N (6-bromo-9*H*-beta-carboline, **2**)[19] and a minor fraction of eudistomin O (8-bromo-9*H*-beta-carboline, **34**).[19] Alkylation of both Br-carbolines **2** and **34** using a series of electrophiles directly afforded the N-alkylated derivatives **23–33** and **35–39** as crystalline compounds in yields between 23% and quantitative (Scheme 1).

The in vitro biological evaluation of nostocarboline (**1**), its derivatives **5–12** and dimers **13–22** was carried out against four parasites, *Trypanosoma brucei rhodesiense* STIB 900, *Trypanosoma cruzi* Tulahuen C2C4, *Leishmania donovani* MHOM-ET-67/L82 axenic amastigotes, and *P. falciparum* K1 strain. The corresponding IC_{50} values are given in Table 1. In addition, cytotoxicity was measured against L6 rat myoblasts and the IC_{50} value as well as the selectivity index ($SI = IC_{50}(L6)/IC_{50}(P.f.)$) are reported in Table 1. In general, nostocarboline (**1**) and the derivatives **5–12** are weakly active against *L. donovani*, *T. brucei* and *T. cruzi*, with the most active being the 3-phenylpropyl substituted compound **12**. This compound displayed single digit micromolar activity against *T. brucei*, and was found to be roughly 3 times less active against the other parasites. In contrast, nostocarboline (**1**) and the derivatives **5–12** are clearly more active against *P. falciparum*, where submicromolar activities were determined for compound **1**, **5** and **6**. The most active compound in this series was the parent nostocarboline (**1**), which showed a pronounced activity of 194 nM. Cytotoxicity of nostocarboline was low, as an IC_{50} value of 120.9 μM was determined, resulting in a SI of 622. In order to study the influence of structure on activity, we calculated a set of molecular descriptors, of which total surface area and clog *P* are reported in Table 1. The relationship of antiplasmodial activity compared to the total



Compound	Residue R	Linker	X	Time [h]	Yield [%]
5	$-C_2H_5$		I	18	43
6	allyl		Br	21	45
7	$-n$Bu		I	overnight	37
8	$-(CH_2)_3COOCH_3$		Br	overnight	24
9	benzyl		Br	15	58
10	*p*-fluoro benzyl		Br	overnight	96
11	*p*-nitro benzyl		Br	overnight	55
12	3-phenyl propyl		Br	overnight	31
13		2-(*Z*)-butene-1,4-diyl	Cl	12	72
14		2-butyn-1,4-diyl	Cl	12	65
15		xylo-1,4-diyl	Cl	12	95
16		xylo-1,3-diyl	Cl	12	89
17		4,4'-bis(methanyl)biphenyl	Cl	12	86
18		bis(ethanyl)ether	Cl	24	60
19		hexan-1,6-diyl	Br	48	93
20		octan-1,8-diyl	Br	48	87
21		decan-1,10-diyl	Br	48	78
22		dodecan-1,12-diyl	Br	48	71
23	$-CH_3$		I	18	71
24	$-C_2H_5$		I	15	83
25	allyl		Br	15	66
26	$-n$Bu		I	15	43
27	$-(CH_2)_3COOCH_3$		Br	15	79
28	benzyl		Br	15	quant.
29	*p*-fluoro benzyl		Br	1	96
30	*m*-fluoro benzyl		Br	22	44
31	*p*-nitro benzyl		Br	5	99
32	3-phenyl propyl		Br	15	94
33	CH_2naphtyl		Br	5	82
35	$-C_2H_5$		I	overnight	23
36	allyl		Br	overnight	24
37	benzyl		Br	overnight	44
38	*p*-fluoro benzyl		Br	overnight	25
39	CH_2naphtyl		Br	overnight	43

Scheme 1. Preparation of nostocarboline derivatives **5–12**, dimers **13–22** and eudistomin derivatives **23–33** and **35–39**.

surface area for nostocarboline (**1**) and derivatives **5–12** reveals an interesting trend (Fig. 2). Clearly, increasing the total surface area by replacing the CH_3 substituent in nostocarboline (**1**) with larger groups results in a loss of antiplasmodial activity while increasing the cytotoxicity of the compounds. This trend is reflected in a selectivity drop, where a change in the SI from 622 to 9 was observed by replacing CH_3 by a Bn group.

The dimeric series **13–22** was generally found to be more active than the nostocarboline derivatives, displaying single digit μM IC_{50} values against *T. cruzi*, and submicromolar values against *L. dono-*

Table 1
Antiparasitic in vitro activities of nostocarboline 1, the derivatives **5**–**12**, the dimers **13**–**22** and the eudistomin derivatives **23**–**33** and **35**–**39**

Compound	Residue R	*L.d.*[a]	*T.b.*[b]	*T.c.*[c]	*P.f.*[d]	Cytotoxicity[e]	SI[f]	Surface[g]	clog *P*[h]
1	$-CH_3$	34.3	70.5	>87.1	0.194	120.9	622	204.97	2.83
5	$-C_2H_5$	251.0	36.8	100.2	0.452	113.0	250	222.48	3.16
6	Allyl	196.1	33.5	57.8	0.831	126.5	152	235.23	3.33
7	–*n*Bu	112.1	11.6	103.8	1.616	74.0	46	254.52	4.09
8	$-(CH_2)_3COOCH_3$	116.6	105.6	114.6	3.143	207.1	66	300.72	3.65
9	Benzyl	112.6	6.2	24.6	2.997	27.0	9	276.2	4.38
10	*p*-Fluoro benzyl	110.5	18.1	87.7	4.672	111.3	24	285.7	4.44
11	*p*-Nitro benzyl	145.6	21.3	32.8	2.209	42.2	19	307.03	4.25
12	3-Phenyl propyl	18.6	6.2	20.9	1.608	71.3	44	310.29	4.90
13	2-*Z*-Butene-1,4-diyl	9.6	1.1	>56.6	0.113	61.1	540	412.14	5.51
14	2-Butyn-1,4-diyl	34.7	6.4	10.6	0.738	28.5	39	409.43	5.42
15	Xylo-1,4-diyl	19.9	1.0	>51.7	0.223	17.4	78	455.58	6.77
16	Xylo-1,3-diyl	0.2	1.2	5.9	0.121	3.8	32	456.21	6.77
17	4,4′-Bis(methanyl)biphenyl	68.4	2.5	>45.7	0.056	23.0	408	527.38	8.45
18	Bis(ethanyl)ether	8.6	1.2	51.1	0.018	47.9	2625	430.05	4.88
19	Hexan-1,6-diyl	6.6	1.2	36.2	0.020	36.2	1810	449.74	6.80
20	Octan-1,8-diyl	2.3	0.9	31.4	0.018	7.5	423	478.10	7.73
21	Decan-1,10-diyl	0.9	1.1	36.6	0.014	8.2	575	510.41	8.66
22	Dodecan-1,12-diyl	0.6	1.2	10.0	0.023	4.3	186	542.17	9.59
23	$-CH_3$	>230	47.2	131.9	0.018	86.1	4783	n.d.	n.d.
24	$-C_2H_5$	>223	26.4	90.5	0.032	78.8	2443	n.d.	n.d.
25	Allyl	20.1	17.4	86.6	0.492	130.9	266	n.d.	n.d.
26	–*n*Bu	35.1	11.7	35.3	1.151	68.7	60	n.d.	n.d.
27	$-(CH_2)_3COOCH_3$	>203	111.0	64.6	2.330	162.3	70	n.d.	n.d.
28	Benzyl	21.2	5.2	16.3	2.368	61.6	26	n.d.	n.d.
29	*p*-Fluoro benzyl	16.4	4.7	13.3	1.761	22.6	13	n.d.	n.d.
30	*m*-Fluoro benzyl	11.0	5.6	25.9	2.153	41.5	19	n.d.	n.d.
31	*p*-Nitro benzyl	38.2	10.8	14.1	1.330	18.1	14	n.d.	n.d.
32	3-Phenyl propyl	20.4	4.5	12.6	0.459	27.5	60	n.d.	n.d.
33	CH_2naphthyl	17.7	4.1	7.5	0.481	5.1	11	n.d.	n.d.
35	$-C_2H_5$	>223	25.0	169.6	6.624	153.4	23	n.d.	n.d.
36	Allyl	123.1	26.2	153.6	6.738	112.2	17	n.d.	n.d.
37	Benzyl	66.5	7.0	42.9	2.583	28.7	11	n.d.	n.d.
38	*p*-Fluoro benzyl	53.4	3.8	28.7	3.279	17.5	5	n.d.	n.d.
39	CH_2naphthyl	46.4	4.4	7.9	0.502	3.7	7	n.d.	n.d.

All results are reported as IC_{50} values in μM.
[a] *Leishmania donovani* MHOM-ET-67/L82.
[b] *Trypanosoma brucei* rhodesiense STIB 900.
[c] *Trypanosoma cruzi* Tulahuen C2C4.
[d] *Plasmodium falciparum* K1.
[e] Rat myoblast L6 cells.
[f] The selectivity index is calculated by IC_{50}((L6)/IC_{50}(*P.f.*)).
[g] The surface in Å^2 was calculated using moloc (http://www.moloc.ch).
[h] *c*Log *P* was calculated using Osiris Property Explorer developed by Thomas Sander from Actelion (Basel). n.d. = not determined.

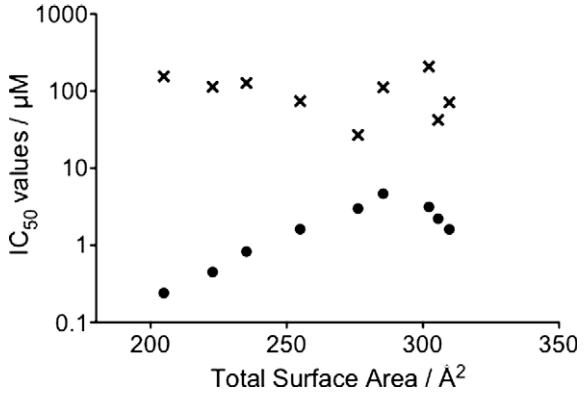


Figure 2. Antiplasmodial activity (●) and cytotoxicity (×) of nostocarboline (**1**) and derivatives **5**–**12** reported as IC_{50} values plotted against the total surface area. Increasing the surface results in decreased activity and decreased selectivity of the compounds.

vani and *T. brucei* (Table 1). Against *L. donovani*, longer and flexible linkers such as in **18–22** gave better IC_{50} values, reaching 0.6 μM for the C_{12} derivative **22**. This represents an increase in activity of 2 orders of magnitude when compared to derivatives involving more restricted linkers such as **17**. However, this trend again appears correlated to cytotoxicity, where also an increase can be found. A drastic impact of structure on activity was found for xylyl derivatives **15** and **16**: While the *para* substituted **15** displays weak activity (IC_{50} = 20 μM), a value of 0.2 μM was found for the corresponding *meta* substituted derivative **16**, giving rise to a two orders of magnitude increase in activity upon change of aromatic substitution from *para* to *meta*. These data suggest that the relative orientation of the carbolinium structures in the dimer **16** directly affects the inhibition of *L. donovani*. Consistent low micromolar values were found against *T. brucei* and there appears to be little influence of linker properties on bioactivity. When compared to these values, activity against *T. cruzi* was decreased 10 to 50-fold, and generally values almost equal or higher to those observed against L6 rat myoblasts were measured, indicating little or no selectivity.

The nostocarboline dimers **13–22** showed very potent activity against *P. falciparum*, and values as low as 14 nM were determined (for compound **21**). Again, dimers employing a long and flexible linker such as **17–22** display better activity, as IC_{50} values below 100 nM were determined. These derivatives, however, also display increased cytotoxicity down to low micromolar values. From the point of selectivity, shorter but flexible linkers are preferred, with **18** and **19** displaying selectivities for *P. falciparum* over L6 myo-

blasts of >2600 and >1800-fold, respectively. Compound **18** proved to be optimal in this series, while displaying very potent activity (18 nM against *P. falciparum*) and a selectivity of >2600 against L6 rat myoblasts. These in vitro data of nostocarboline dimers **13–22** complement earlier studies on bis cationic dimers, a class of compounds known for antiprotozoal activity.[26]

The eudistomin derivatives **23–33** and **35–39** were evaluated in vitro against the same four parasites: *L. donovani*, *T. brucei rhodesiense*, *T. cruzi* and *P. falciparum*. These results and also the cytotoxicity against rat myoblast L6 cells and the selectivity index (SI) are reported in Table 1. From the 6-bromo derivatives **23–33**, the 2-naphthyl substituted compound **33** displayed the strongest activity against *L. donovani*, *T. brucei* and *T. cruzi* with activities of 17.7 μM, 4.1 μM and 7.5 μM, respectively. Similar results were obtained for the 8-bromo derivatives **35–39**, with the 2-naphthyl substituted compound **39** displaying the strongest activities of 46.4 μM, 4.4 μM and 7.9 μM against the same three parasites. In contrast, stronger activities against *P. falciparum* were observed with IC_{50} values reaching 18 nM and 32 nM for the 6-bromo derivatives **23** (R = Me) and **24** (R = Et), respectively. In addition, these two compounds exhibit a low cytotoxicity of 86.1 μM and 78.8 μM, resulting in a very high selectivity index of 4783 and 2443, respectively. Again, as discussed for the Cl-series above, for both 6-bromo derivatives **23–33** and 8-bromo derivatives **35–39**, it was evident that increasing the size of the residue on the pyridine nitrogen caused a loss of activity and an increase in cytotoxicity. This trend directly influenced also the SI value, as for example it drastically passed from 4783 for the methyl derivative **23** to 11 for the 2-naphthyl derivative **33**. It is interesting also to compare the compounds **24** and **35**; both compounds had the ethyl group as residue, but different positioning of the Br group on the carboline. Compound **24** with the bromine at C(6) displayed an IC_{50} values of 32 nM, while compound **35** with the bromine at C(8) displayed an IC_{50} values of 6.6 μM, resulting in a loss of activity of more than 200-fold. This difference in activity disappeared of the 6-bromo and 8-bromo derivatives with the same residue, but of larger size. In general high cytotoxicity was again observed for large substituents, and in particular for phenyl or naphthyl groups, that is, compound **33** (IC_{50} = 5.1 μM) or **39** (IC_{50} = 3.7 μM). This is in line with the Cl-series discussed above and can be explained by similar factors.

The observed values of these simple quaternary carbolinium alkaloids compare well with literature precedents (Fig. 3). For example, normelinonine F (or deschloronostocarboline) was found to display an IC_{50} value of 13.6 μM and no cytotoxicity was reported.[20a] A more recent study measured an IC_{50} value of 0.45 μM against the K1 strain.[20b] Interestingly, addition of a Cl substituent (to give **1**) at the 6-position led to an increase of activity (Table 1 and Fig. 2). Tetra- and pentacyclic anhydronium bases such as for example fascaplysin,[21] cryptolepine[20,22] or isoneocryptolepine[23] display similar inhibition of the chloroquine resistant K1 strain of *P. falciparum*, yet with increased cytotoxicity. Literature reports attribute these cytotoxicity issues to DNA intercalation properties, and cryptolepine was found to be a potent DNA intercalator, topoisomerase II inhibitor and cytotoxic agent.[22b] In addition, isoneocryptolepine was found active in a DNA-methyl green assay based on improved DNA intercalation.[23] These results thus substantiate the hypothesis that larger lipophilic cations such as cryptolepine or the aryl nostocarboline derivatives **9–12** or aryl eudistomin N derivatives **29–33** display unselective cytotoxicity which might be due to unfavorable interaction with DNA. Along these lines, so-called π-delocalized lipophilic cations (DLC) have been shown to be powerful cytotoxic agents and antimalarial agents.[24] However, the observation that increased surface area results in increased cytotoxicity is also supported by these studies.[24] This is also corroborated by reports on manzamine, where quaternization results in increased activity, although with increased cytotoxicity.[25]

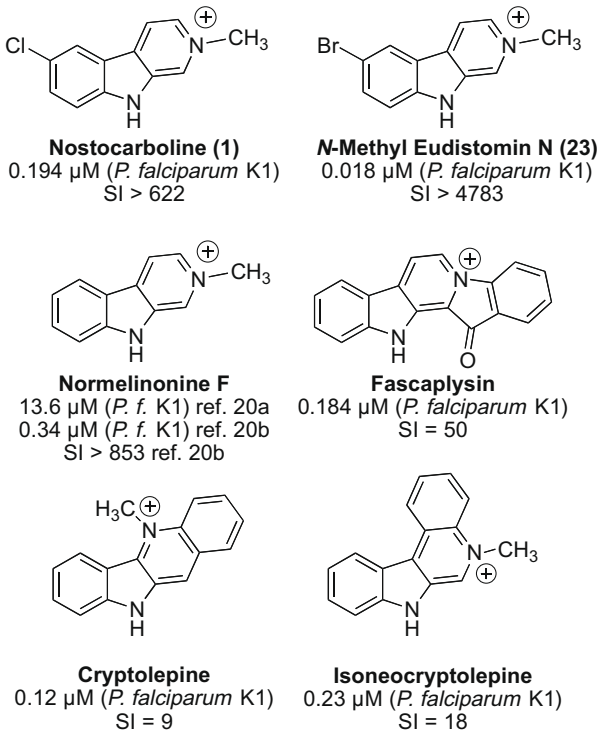


Figure 3. Anhydronium bases such as normelinonine F,[20] fascaplysin,[21] cryptolepine[20,22] and isoneocryptolepine[23] display antiplasmodial activity and pronounced cytotoxicity when compared to nostocarboline (**1**).

Nostocarboline (**1**), its ethyl derivative **5**, four dimers **13**, **17–19** and the eudistomin derivatives **23–25** that showed potent and selective in vitro activity were selected for in vivo evaluation in a *P. berghei* mouse model (Table 2). Compounds were administered intraperitoneally as DMSO/water formulations on four consecutive days (4, 24, 48 and 72 h post infection). Parasitaemia was determined on day 4 post infection (24 h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean per cent parasitaemia for the control (n = 5 mice) and treated groups expressed as a per cent relative to the control group. As can be seen in Table 2, the dimers **13** and **17–19** and the eudistomin derivatives **23–25** were found to be inactive in vivo. Interestingly, nostocarboline (**1**) displayed roughly a 50% reduction in parasitaemia when compared to the control group at a dose of 50 mg/kg, and the mean survival time increased from 6.3 to 7.3 d. No toxicity

Table 2
Biological in vivo evaluation of nostocarboline (**1**), ethyl derivative **5**, dimers **13**, **17–19**, and eudistomin derivatives **23–25** in the *P. berghei* mouse model

Compound	Dose (mg/kg)	Route	% Activity[a]	Survival (d)
Nostocarboline (**1**)	4 × 50	i.p.	49.6	7.3
5	4 × 50	i.p.	15.36	n.d.
13	4 × 50	i.p.	11.53	n.d.
17	4 × 50	i.p.	0	n.d.
18	4 × 50	i.p.	2.6	n.d.
19	4 × 50	i.p.	0	n.d.
23	4 × 50	i.p.	0	n.d.
24	4 × 50	i.p.	13.25	n.d.
25	4 × 50	i.p.	3.57	n.d.
Control				6.3

The compounds were formulated in DMSO/water.
[a] Activity means reduction of parasitaemia versus the untreated control group n.d. = not determined due to inactivity, mice euthanized on day 4 (for **1**, **13–19**) or on day 6 (for **5**, **23–25**) after parasitaemia determination.

Table 3
Antitubercular evaluation of nostocarboline (**1**) and dimers **13** and **15–22**. MIC_{99} values are given in μg/ml and in μM in brackets

Compound	MIC_{99} *M. tuberculosis* H37Rv	MIC_{99} *M. smegmatis* mc^2155	MIC_{99} *C. glutamicum* ATCC13032
13	5 [9]	>100 [>188]	2.5 [5]
15	>10 [>17]	>100 [>170]	>10 [>17]
16	10 [20]	25 [50]	10 [20]
17	>10 [>15]	100 [152]	>10 [>15]
18	10 [18]	25 [46]	5 [9]
19	5 [8]	12.5 [20]	5 [8]
20	5 [7]	100 [147]	5 [7]
21	2.5 [4]	>100 [>142]	10 [14]
22	5 [7]	12.5 [17]	10 [14]
Nostocarboline (**1**)	>10 [>29]	100 [290]	>10 [>29]

was observed for all compounds at a dose of 50 mg/kg. In earlier experiments, isoneocryptolepine was found to suppress parasitaemia by 38.9% (50 mg/kg s.c.)[23] and cryptolepine was found to be toxic at a dose of 20 mg/kg ip,[22c] although the route of administration was found to be relevant,[22a] as earlier studies demonstrated some efficacy in po administration at similar doses.[20]

The biological profile of nostocarboline (**1**) and the dimers **13–22** was further complemented by their biological in vitro evaluation against *M. tuberculosis* H37Rv, *Mycobacterium smegmatis* mc^2155 and *Corynebacterium glutamicum* ATCC13032 (Table 3). Nostocarboline (**1**) was not found active against these three strains, which is in line with an earlier study demonstrating no activity against several strains of *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Streptococcus*, *Haemophilus*, *Moraxella and Escherichia*.[17a] In contrast, several dimers were active against *M. tuberculosis*, and a MIC_{99} value of 2.5 μg/ml was found for dimer **21** featuring a C_{10} linker. Dimers with different alkyl linkers were found active too, although with a slightly reduced activity of 5 μg/ml.

3. Conclusion

In conclusion, we have reported the synthesis and biological evaluation of nostocarboline (**1**), its derivatives **5–12**, dimers **13–22** and eudistomin derivatives **23–33** and **35–39** against four parasites and three bacteria relevant to tuberculosis research. While some nostocarboline dimers displayed potent antiplasmodial activity in vitro, very weak or no activity was observed in the *P. berghei* mouse model. The best compound was the parent natural product, nostocarboline (**1**). Benefits of this natural product include (1) simple, inexpensive and straightforward preparation (2) potent and selective in vitro activity against *P. falciparum* K1 and (3) moderate in vivo activity in the *P. berghei* mouse model. Based on the structure/activity relationships reported in this study, as well as literature precedents, we think that smaller carboline anhydronium bases **3** or their salts **1** display favorable properties and reduced cytotoxicity (due to decreased DNA intercalation). These conclusions should help in the design of more efficient carbolinium anhydronium bases as antiplasmodial agents.

4. Experimental section

Solvents and reagents were purchased reagent-grade and used without further purification, unless noted otherwise. 6-Chloro-norharmane was prepared via tetrahydrocarboline[27a] in a modification of the procedure reported by Nakano.[27b] Solvents for extractions and recrystallizations were of analytical grade and used as received. Deuterated solvents were obtained from Armar Chemicals, Switzerland. All reactions were carried out under an atmosphere of Ar or N_2 and magnetically stirred unless otherwise stated. Yields refer to recrystallized or chromatographically purified, spectroscopically pure compounds. Melting points (mp) were determined on a Büchi 545 apparatus in open glass capillaries and are uncorrected. ^{1}H NMR spectra were recorded on Bruker DPX 400 MHz spectrometer or on Bruker Avance 500 MHz spectrometer at 298 K in the indicated deuterated solvent. Data are reported as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet or not resolved signal; br, broad signal), coupling constants (J, Hz), integration. All signals were referenced to the internal solvent signal as standard (CD_3OD, δ 3.34). IR spectra were recorded on a Varian 800 FT-IR ATR spectrometer and data are reported in terms of frequency of absorption (ν, cm^{-1}) with corresponding characteristic intensity (w, weak; m, medium; s, strong; br, broad signal). Mass spectra were obtained from the Mass Spectroscopy Service from the EPFL. High-resolution measurements were recorded on an IonSpec 4.7 FT-ICR (ESI) using the DHB (2,5-Dihydroxy-benzoic acid) matrix or on a MICROMASS (ESI) Q-TOF Ultima API (Waters Corporation, Milford, MA, USA). Data are reported as mass-to-charge ratio (m/z). Analytical RP-HPLC was performed on a Dionex HPLC System (Interface ASI-100 Chromeleon, UV detector PDA-100, Pump P-680, degaser). The flow rate was 1 mL/min and the detector was fixed at the indicated wavelength. Column: Phenomenex Gemini® RP-18 (5 μm, 150 mm–4.6 mm), solvent A: CH_3CN, solvent B: 0.1% TFA in H_2O. Conditions and retention times (t_R) are indicated. All separations were performed at ambient temperature.

4.1. X-ray crystal structure determination

The Data collections were performed at low temperature using Mo Kα radiation on an Oxford Diffraction/Varian Sapphire/KM4 CCD, (**3a**, **23**) and on a Bruker/Nonius APEX II CCD (**25**), both having a kappa geometry goniometer. Data were reduced by means of Crysalis PRO[28] (**3a**, **23**) and EVALCCD[29] and then corrected for absorption.[30] The solutions and refinements were performed by SHELX.[31] The crystal structures were refined using full-matrix least-squares on F^2 with all non hydrogen atoms anisotropically defined. Hydrogen atoms were placed in calculated positions by means of the 'riding' model. A twinning problem was discovered during the refinement of **23** and treated by means of the TWINROTMAT routine found in PLATON.[32] A new dataset was then created and used in the final stages of refinement with cards HKLF 5, MERG 0 and one refined BASF parameter [0.171(4)].

4.1.1. 6-Chloro-2-methyl-2*H*-beta-carboline (3a, nostocarboline anhydronium base)

To a mixture of nostocarboline (**1**) (33.0 mg, 0.096 mmol, 1.00 equiv) in EtOAc (15.0 mL), a solution of NaOH (1 M, 7.5 mL) was added dropwise. The starting material immediately dissolved and generated a strongly yellow colored mixture that was stirred at rt for 10 min. The mixture was extracted with EtOAc (3×) carefully recovering only the organic phases. The combined organic phases could not be dried using standard salts ($NaSO_4$ or $MgSO_4$) without reprotonation of the generated base and they were directly concentrated and dried under high vacuum to afford the anhydronium base **3a** (18.1 mg, 0.084 mmol, 88%) as a yellow solid. An analytical sample was recrystallized ($MeOH/Et_2O$/hexane) for X-ray analysis. ^{1}H NMR (400 MHz, CD_3OD) δ 8.79 (s, 1H), 8.25 (d, J = 6.2 Hz, 1H), 8.17 (d, J = 2.1 Hz, 1H), 7.92 (dd, J_1 = 6.5 Hz, J_2 = 1.2 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.49 (dd, J_1 = 8.8 Hz, J_2 = 2.1 Hz, 1H), 4.38 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 179.0, 153.6, 144.4, 131.6, 129.2, 126.3, 123.3, 121.4, 121.1, 118.2, 115.9, 46.2; HR-MS (ESI) calcd for $C_{12}H_{10}ClN_2$: $[M+H]^+$ 217.0533; found 217.0525; FTIR (ATR) ν 3005w, 2932w, 2855w, 1570s, 1408s, 1335m, 1285m, 1246m, 1157m, 1092w, 1053m, 1015m, 922m, 872w, 806m, 783m, 752m, 702m cm^{-1}.

4.1.2. Crystallographic details for compound 3a

A total of 8596 reflections ($-7 \leqslant h \leqslant 7, -28 \leqslant k \leqslant 27, -8 \leqslant l \leqslant 9$) were collected at $T = 140(2)$ K in the range of 3.03–26.36° of which 1978 were unique ($R_{int} = 0.0795$); Mo Kα radiation ($\lambda = 0.71073$ Å). The residual peak and hole electron densities were 0.400 and −0.411 eÅ^{-3}, respectively. The absorption coefficient was 0.354 mm^{-1}. The least squares refinement converged normally with residuals of $R(F) = 0.0644$ (observed data), $wR(F^2) = 0.1456$ (all data) and a GoF = 1.000. $C_{12}H_9ClN_2$, $M_w = 216.66$, space group $P2_1/c$, monoclinic, $a = 6.0420(8)$, $b = 22.907(3)$, $c = 7.2662(11)$ Å, $\beta = 104.655(14)°$, $V = 973.0(2)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.479$ Mg/m^3. CCDC-728844 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.1.3. 6-Chloro-2-ethyl-9*H*-beta-carbolin-2-ium iodide (5)

To a mixture of 6-chloro-norharmane (**4**) (500 mg, 2.47 mmol, 1.00 equiv) in CH_3CN (15 mL) was added ethyl iodide (490 μL, 6.18 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 18 h. The reaction was concentrated; then the residue was dissolved in a minimum amount of CH_3CN, the product precipitated by addition of Et_2O, collected by filtration and washed with a mixture of CH_3CN/Et_2O. The product was dissolved in MeOH and any precipitate was removed by filtration. The filtrate was concentrated and dried under high vacuum affording compound **5** (384 mg, 1.07 mmol, 43%) as a crystalline brown solid. Mp = 215.0–216.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.38 (s, 1H), 8.74 (d, $J = 6.8$ Hz, 1H), 8.66 (dd, $J_1 = 6.8$ Hz, $J_2 = 1.2$ Hz, 1H), 8.51–8.50 (m, 1H), 7.81–7.80 (m, 2H), 4.85 (q, $J = 7.2$ Hz, 2H), 1.77 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 142.9, 136.1, 132.3, 132.3, 132.1, 129.3, 127.3, 122.4, 120.6, 118.2, 113.8, 56.9, 16.0; HR-MS (ESI) calcd. for $C_{13}H_{12}ClN_2$: $[M]^+$ 231.0689; found 231.0692; FTIR (ATR) ν 3418w, 3098m, 3017m, 2288w, 1647m, 1570w, 1489s, 1447s, 1319m, 1281s, 1250m, 1169m, 1142s, 1065s, 937w, 876m, 806s, 725m, 706m cm^{-1}.

4.1.4. 2-Allyl-6-chloro-9*H*-beta-carbolin-2-ium bromide (6)[17a]

To a solution of 6-chloro-norharmane (**4**) (50.0 mg, 0.25 mmol, 1.00 equiv) in *i*PrOH (4.0 mL) was added allyl bromide (43 μL, 0.50 mmol, 2.00 equiv). The flask was sealed and heated at 85 °C for 21 h. The reaction was concentrated; then the residue was dissolved in a minimum amount of CH_3CN, the product precipitated by addition on Et_2O, collected by filtration and washed with a mixture of CH_3CN/Et_2O. The product was dissolved in MeOH and any precipitate was removed by filtration. The filtrate was concentrated and dried under high vacuum affording compound **6** (36.0 mg, 0.11 mmol, 45%) as a crystalline solid. Mp = 174–177 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 9.31 (s, 1H), 8.71 (d, $J = 6.6$ Hz, 1H), 8.58 (dd, $J_1 = 6.6$ Hz, $J_2 = 1.1$, 1H), 8.47 (d, $J = 1.1$ Hz, 1H), 7.78 (s, 2H), 6.33–6.20 (m, 1H), 5.55 (d, $J = 1.4$ Hz, 1H), 5.51 (d, $J = 5.1$ Hz, 1H), 5.39 (d, $J = 6.3$ Hz, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 143.5, 136.7, 133.3, 133.2, 133.1, 132.3, 130.4, 128.0, 123.2, 122.4, 121.2, 119.0, 114.9, 64.1; MS 245.06 (16), 243.06 (100), 209.10 (6); HR-MS (MALDI) Anal. Calcd for $C_{14}H_{12}ClN_2$: $[M]^+$ 243.0689; found 243.0680; FTIR (ATR) ν 3007m, 2949m, 2855m, 1905w, 1813w, 1745s, 1651s, 1489s, 1285s, 951s, 806s. RP-HPLC: $t_R = 16.79$ min (C_{18}, 5–95% A in 30 min; 95% A for 3 min, 95–5% A in 1 min).

4.1.5. 2-Butyl-6-chloro-9*H*-beta-carbolin-2-ium iodide (7)

To a solution of 6-chloro-norharmane (**4**) (30.0 mg, 0.15 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added iodobutane (42 μL, 0.37 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was concentrated and then the residue was triturated using a mixture of Et_2O/CH_3CN and the precipitate collected by filtration. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrate was concentrated and dried under high vacuum affording compound **7** (21.2 mg, 0.055 mmol, 37%) as a crystalline solid. Mp = 213.0–214.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.37 (s, 1H), 8.73 (d, $J = 6.8$ Hz, 1H), 8.64 (d, $J = 6.4$ Hz, 1H), 8.51 (s, 1H), 7.81–7.80 (m, 2H), 4.80 (t, $J = 7.5$ Hz, 2H), 2.12 (quint., $J = 7.5$ Hz, 2H), 1.49 (sext., $J = 7.5$ Hz, 2H), 1.06 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.0, 136.2, 132.3, 132.3, 132.2, 129.5, 127.3, 122.4, 120.6, 118.1, 114.1, 61.3, 33.6, 19.2, 12.5; HR-MS (ESI) calcd for $C_{15}H_{16}ClN_2$: $[M]^+$ 259.1002; found 259.0999; FTIR (ATR) ν 3445w, 3032s, 2997s, 2959s, 2858m, 1651m, 1570w, 1516m, 1489s, 1450s, 1323m, 1281s, 1165m, 1142s, 1065s, 903w, 872m, 806s, 756m, 725s cm^{-1}.

4.1.6. 6-Chloro-2-(4-methoxycarbonyl-butyl)-9*H*-beta-carbolin-2-ium bromide (8)

To a solution of 6-chloro-norharmane (**4**) (30.0 mg, 0.15 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added methyl bromovalerate (53 μL, 0.37 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was concentrated; then the residue was triturated in a mixture of Et_2O/CH_3CN and the precipitate was collected by filtration. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording compound **8** (14.5 mg, 0.036 mmol, 24%) as a crystalline solid. Mp = 159.0–160.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.36 (s, 1H), 8.74 (d, $J = 6.4$ Hz, 1H), 8.64 (d, $J = 6.0$ Hz, 1H), 8.53 (s, 1H), 7.83 (s, 2H), 4.81–4.78 (m, 2H), 3.68 (s, 3H), 2.48 (t, $J = 7.2$ Hz, 2H), 2.19–2.13 (m, 2H), 1.76–1.72 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.8, 143.1, 136.2, 132.4, 132.3, 129.6, 127.4, 122.4, 120.6, 118.1, 114.1, 61.0, 50.7, 32.4, 30.8, 21.1; HR-MS (ESI) calcd for $C_{17}H_{18}ClN_2O_2$: $[M]^+$ 317.1057; found 317.1062; FTIR (ATR) ν 3426w, 3036w, 2994w, 2951m, 2905w, 1728s, 1647m, 1570w, 1520m, 1493m, 1439m, 1350m, 1281s, 1227m, 1157s, 1126s, 1069s, 984s, 891m, 810s, 752s cm^{-1}.

4.1.7. 2-Benzyl-6-chloro-9*H*-beta-carbolin-2-ium bromide (9)[17a]

To a solution of 6-chloro-norharmane (**4**) (50.0 mg, 0.25 mmol, 1.00 equiv) in *i*PrOH (8.0 mL) was added benzyl bromide (60 μL, 0.50 mmol, 2.00 equiv). The flask was sealed and heated at 85 °C for 15 h. The reaction was concentrated; then the residue was dissolved in a minimum amount of CH_3CN, the product precipitated by addition of Et_2O, collected by filtration and washed with a mixture CH_3CN/Et_2O. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording (**9**) (54.0 mg, 0.14 mmol, 58%) as a crystalline solid. Mp = 121–125 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 9.41 (*s*, 1H), 8.71–8.67 (*m*, 2H), 8.45 (*s*, 1H), 7.89 (*s*, 1H), 7.76 (*d*, $J = 1.25$, 2H), 7.55–7.44 (*m*, 5H), 5.96 (*s*, 2H); ^{13}CNMR (150 MHz, CD_3OD) δ 143.2, 136.4, 135.2, 133.3, 132.9, 130.3, 130.2, 130.2, 129.7, 127.8, 123.0, 120.8, 119.1, 114.7, 79.2, 65.1; LR-MS 295.08 (37), 293.08 (100), 259.12 (53); HR-MS (MALDI) Anal. Calcd for $C_{18}H_{14}ClN_2$: $[M]^+$ 293.0846; found 293.0840; FTIR (ATR) 3399w, 3057m, 2970m, 1643m 1491s, 1284s, 730s; RP-HPLC: $t_R = 19.35$ min (C_{18}, 5–95% A in 30 min; 95% A for 3 min, 95–5% A in 1 min).

4.1.8. 6-Chloro-2-(4-fluoro-benzyl)-9*H*-beta-carbolin-2-ium bromide (10)

To a solution of 6-chloro-norharmane (**4**) (30.0 mg, 0.15 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added 4-fluorobenzyl bromide (69 μL, 0.37 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was concentrated; then the residue triturated using a mixture of Et_2O/CH_3CN and the precipitate collected by filtration. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrate was concentrated and dried under high vacuum affording compound **10** (56.3 mg,

0.14 mmol, 96%) as a crystalline solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.50 (s, 1H), 8.75 (d, J = 6.8 Hz, 1H), 8.71 (dd, J_1 = 6.8 Hz, J_2 = 1.6 Hz, 1H), 8.51 (t, J = 1.2 Hz, 1H), 7.82 (d, J = 1.2 Hz, 2H), 7.67 (d, J = 5.2 Hz, 1H), 7.65 (d, J = 5.2 Hz, 1H), 7.23 (t, J = 8.7 Hz, 2H), 6.00 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 163.4 (d, J = 248.4 Hz), 143.1, 136.1, 132.6, 132.5, 132.4, 130.9 (d, J = 9.7 Hz), 130.4 (d, J = 3.7 Hz), 129.6, 127.4, 122.5, 120.5, 118.3, 116.0 (d, J = 22.0 Hz), 114.2, 63.2; HR-MS (ESI) calcd for $C_{18}H_{13}ClFN_2$: $[M]^+$ 311.0751; found 311.0738; FTIR (ATR) ν 3460w, 3414w, 3044m, 2982m, 2893w, 1647m, 1605m, 1570w, 1512m, 1489m, 1454m, 1350w, 1281m, 1223m, 1161s, 1119s, 1069s, 883w, 826s, 779m, 760m cm^{-1}.

4.1.9. 6-Chloro-2-(4-nitro-benzyl)-9*H*-beta-carbolin-2-ium bromide (11)

To a solution of 6-chloro-norharmane (**4**) (30.0 mg, 0.15 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added 4-nitrobenzyl bromide (80.0 mg, 0.37 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was concentrated; then the residue triturated using a mixture of Et_2O/CH_3CN and the precipitate was collected by filtration. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrate was concentrated and dried under high vacuum affording (**11**) (34.6 mg, 0.083 mmol, 55%) as a crystalline solid. Mp = 210.0–211.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.55 (s, 1H), 8.80 (d, J = 6.8 Hz, 1H), 8.74 (d, J = 6.8 Hz, 1H), 8.54 (s, 1H), 8.34 (d, J = 8.7 Hz, 2H), 7.84 (s, 2H), 7.76 (d, J = 8.7 Hz, 2H), 6.17 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 144.6, 143.2, 141.2, 136.2, 132.8, 132.7, 132.7, 130.1, 129.3, 127.6, 124.0, 122.6, 120.5, 118.5, 114.2, 62.8; HR-MS (ESI) calcd for $C_{18}H_{13}ClN_3O_2$: $[M]^+$ 338.0696; found 338.0686; FTIR (ATR) ν 3387w, 3059m, 3009m, 1647m, 1609m, 1574w, 1520s, 1493s, 1454m, 1342s, 1285s, 1161m, 1130m, 1069m, 1018w, 856m, 806s, 733s, 710m cm^{-1}.

4.1.10. 6-Chloro-2-(3-phenyl-propyl)-9*H*-beta-carbolin-2-ium bromide (12)

To a solution of 6-chloro-norharmane (**4**) (30.0 mg, 0.15 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added 1-bromo-3-phenylpropane (56 μL, 0.37 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was concentrated; then the residue triturated using a mixture of Et_2O/CH_3CN and the precipitate collected by filtration. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrate was concentrated and dried under high vacuum affording compound **12** (18.7 mg, 0.047 mmol, 31%) as a crystalline solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.31 (s, 1H), 8.68 (d, J = 6.4 Hz, 1H), 8.60 (d, J = 6.4 Hz, 1H), 8.49 (t, J = 1.2 Hz, 1H), 7.81 (d, J = 1.2 Hz, 2 H), 7.25 (s, 2H), 7.24 (s, 2H), 7.13–7.09 (m, 1H), 4.83 (t, J = 7.2 Hz, 2H), 2.83 (t, J = 7.2 Hz, 2H), 2.51–2.45 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.2, 140.1, 136.2, 132.3, 132.2, 129.7, 128.2, 128.0, 127.2, 125.9, 122.3, 120.6, 118.0, 114.2, 61.2, 32.6, 32.1, 22.7; HR-MS (ESI) calcd. for $C_{20}H_{18}ClN_2$: $[M]^+$ 321.1158; found 321.1146; FTIR (ATR) ν 3418w, 3024m, 2990m, 2943m, 2905m, 2843w, 1643m, 1574s, 1520m, 1493s, 1450s, 1412s, 1319m, 1285s, 1157s, 1123s, 1069s, 922m, 876m, 826s, 741m cm^{-1}.

4.2. General procedure 1 (GP 1) for dimerization

To a solution of 6-chloro-norharmane (**4**) (50.0 mg, 0.25 mmol, 2.00 equiv) in *i*PrOH (4 mL, 62.5 mM) was added the bis-halogeno linker (0.125 mmol, 1.00 equiv) at 50 °C and the resulting suspension was heated to reflux for 12–48 h. The resulting yellow suspension was cooled to rt and filtered. The filtrate was washed with ice-cooled *i*PrOH and dried under high vacuum. Purification by recrystallization gave the desired bis-β-carbolinium compound.

4.2.1. 2-(*Z*)-Butene-1,4-diyl homo-dimer (13)

(*Z*)-1,4-Dichlorobut-2-ene (14 μL, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **13** (48 mg, 0.090 mmol, 72%). Yellow powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.50 (br s, 2H), 8.78 (d, J = 6.5 Hz, 2H), 8.73 (d, J = 6.5 Hz, 2H), 8.51 (br s, 2H), 7.81 (m, 4H), 6.30 (br s, 2H), 5.81 (br s, 4H); HR-MS (ESI) Anal. calcd for $C_{26}H_{20}Cl_2N_4$: $[M]^{++}$ 230.0605; found: 230.0603; FTIR (ATR) ν 3056w, 3011w, 2964w, 2909w, 2865w, 2782w, 1649s, 1573m, 1520s, 1492s, 1453s, 1376w, 1320m, 1280s, 1253m, 1219w, 1170w, 1158w, 1133s, 1071s, 1023w, 963m, 919w, 882m, 828s, 806s, 782s, 704s, 652w, 620s; RP-HPLC: t_R = 13.27 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 1 min).

4.2.2. 2-Butyn-1,4-diyl homo-dimer (14)

1,4-Dichlorobut-2-yne (13 μL, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **14** (43 mg, 0.081 mmol, 65%). Dark brown powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.15 (br s, 2H), 8.64 (br s, 2H), 8.38 (br s, 2H), 8.22 (br s, 2H), 7.71 (br s, 4H), 3.35 (br s, 4H); HR-MS (ESI) Anal. calcd for $C_{26}H_{18}Cl_2N_4$: $[M]^{++}$ 229.0527; found: 229.0525; FTIR (ATR) ν 3032w, 2928w, 2817w, 2712w, 1642s, 1570w, 1487s, 1452s, 1362w, 1321m, 1280s, 1247w, 1151m, 1069s, 1024w, 886m, 810s, 732m, 607w; RP-HPLC: t_R = 13.19 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 1 min).

4.2.3. Xylo-1,4-diyl homo-dimer (15)

1,4-Bis-chloro-methylbenzene (22 mg, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **15** (69 mg, 0.119 mmol, 95%). Highly hygroscopic yellow powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.43 (br s, 2H), 8.68 (d, J = 6.5 Hz, 2H), 8.66 (d, J = 6.5 Hz, 2H), 8.45 (br s, 2H), 7.76 (br s, 4H), 7.63 (br s, 4H), 5.99 (br s, 4H); HR-MS (ESI) Anal. calcd for $C_{30}H_{22}Cl_2N_4$: $[M]^{++}$ 255.0684; found: 255.0684; FTIR (ATR) ν 3352br, 3053w, 3002w, 2956w, 2900w, 2848w, 2757w, 2651w, 1643s, 1571m, 1516m, 1490s, 1453s, 1403w, 1318s, 1282s, 1253s, 1213m, 1162s, 1124s, 1069s, 1024w, 945w, 875m, 813s, 747s, 705w, 628m; RP-HPLC: t_R = 16.56 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 1 min).

4.2.4. Xylo-1,3-diyl homo-dimer (16)

1,3-Bis-chloro-methylbenzene (22 mg, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **16** (65 mg, 0.111 mmol, 89%). Highly hygroscopic yellow powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.44 (br s, 1H), 9.40 (br s, 1H), 8.72 (d, J = 6.5 Hz, 1H), 8.67 (m, 2H), 8.63 (d, J = 6.5 Hz, 1H), 8.49 (br s, 1H), 8.44 (br s, 1H), 7.76 (br s, 4H), 7.63 (br s, 4H), 5.99 (br s, 4H); HR-MS (ESI) Anal. Calcd for $C_{30}H_{22}Cl_2N_4$: $[M]^{++}$ 255.0684; found: 255.0682; FTIR (ATR) ν 3141w, 3037w, 3004w, 2956w, 2906w, 2852w, 2761w, 2708w, 2657w, 1644s, 1572m, 1517w, 1491s, 1453s, 1321s, 1282s, 1253m, 1222w, 1162m, 1126m, 1070m, 1026m, 874m, 812s, 749s, 708s, 636s; RP-HPLC: t_R = 16.44 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 1 min).

4.2.5. 4,4′-Bis(methanyl)biphenyl homo-dimer (17)

4,4′-Bis(chloromethyl)-1,1′-biphenyl (31 mg, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **17** (71 mg, 0.107 mmol, 86%). Highly hygroscopic yellow powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.43 (br s, 2H), 8.72 (d, J = 6.5 Hz, 2H), 8.69 (d, J = 6.5 Hz, 2H), 8.49 (br s, 2H), 7.79 (m, 4H), 4.78 (br s, 4H), 2.14 (br s, 4H), 1.55 (br s, 4H); HR-MS (ESI) Anal. calcd for $C_{36}H_{26}Cl_2N_4$: $[M]^{++}$ 293.0840; found: 293.0846; FTIR (ATR) ν 3339w, 3049w, 3004w, 2955w, 2902w, 2849w, 2758w, 2704w, 2647w, 1646s,

1614w, 1571m, 1519m, 1492s, 1454s, 1404m, 1318s, 1284s, 1253m, 1211w, 1164m, 1122m, 1068s, 1006s, 946w, 874w, 813s, 756s, 706m, 645s; RP-HPLC: t_R = 18.23 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 1 min).

4.2.6. Bis(ethanyl)ether homo-dimer (18)

Bis(2-chloroethyl)ether (16 μL, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **18** (41 mg, 0.075 mmol, 60%). Yellow powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.30 (br s, 2H), 8.70 (d, J = 6.3 Hz, 2H), 8.62 (d, J = 6.3 Hz, 2H), 8.49 (br s, 2H), 7.78 (m, 4H), 4.95 (m, 4H), 4.06 (m, 4H); HR-MS (ESI) Anal. calcd for $C_{26}H_{22}Cl_2N_4O$: $[M]^{++}$ 239.0658; found: 239.0651; FTIR (ATR) ν 3063m, 2925m, 2855m, 2763w, 1732w, 1647s, 1564s, 1518m, 1488s, 1454s, 1377w, 1326m, 1280s, 1245s, 1165w, 1117s, 1069s, 1037w, 838w, 872m, 816s, 735s, 705m, 663w; RP-HPLC: t_R = 15.97 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 1 min).

4.2.7. Hexan-1,6-diyl homo-dimer (19)

1,6-Dibromo-hexane (19 μL, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **19** (75 mg, 0.116 mmol, 93%). Yellow powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.39 (br s, 2H), 8.68 (d, J = 6.5 Hz, 2H), 8.63 (d, J = 6.5 Hz, 2H), 8.47 (br s, 2H), 7.78 (br s, 4H), 4.78 (br s, 4H), 2.14 (br s, 4H), 1.55 (br s, 4H); HR-MS (ESI) Anal. calcd for $C_{28}H_{26}Cl_2N_4$: $[M]^{++}$ 245.0840; found: 245.0845; FTIR (ATR) ν 3016w, 2987w, 2936w, 2884w, 2834w, 2764w, 2697w, 2647w, 1646s, 1571m, 1519m, 1488s, 1454s, 1320s, 1282s, 1247m, 1155s, 1067s, 1019w, 953w, 866m, 826s, 761w, 736s, 678w, 633s, 609w; RP-HPLC: t_R = 16.47 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 1 min).

4.2.8. Octan-1,8-diyl homo-dimer (20)

1,8-Dibromo-octane (23 μL, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **20** (74 mg, 0.108 mmol, 87%). Yellow powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.34 (br s, 2H), 8.69 (d, J = 6.5 Hz, 2H), 8.61 (d, J = 6.5 Hz, 2H), 8.49 (br s, 2H), 7.79 (br s, 4H), 4.75 (br s, 4H), 2.10 (br s, 4H), 1.45 (br s, 8 H); HR-MS (ESI) Anal. calcd for $C_{30}H_{30}Cl_2N_4$: $[M]^{++}$ 259.0997; found: 259.0996; FTIR (ATR) ν 3009w, 2921m, 2851m, 2764w, 2700w, 2652w, 1646s, 1572m, 1518m, 1491s, 1453s, 1401w, 1352w, 1318s, 1281s, 1253w, 1169w, 1155w, 1129m, 1072s, 1024w, 952w, 856m, 815s, 734m, 631s; RP-HPLC: t_R = 16.95 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 1 min).

4.2.9. Decan-1,10-diyl homo-dimer (21)

1,10-Dibromo-decane (28 μL, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **21** (69 mg, 0.097 mmol, 78%). Light yellow powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.33 (br s, 2H), 8.69 (d, J = 6.5 Hz, 2H), 8.61 (d, J = 6.5 Hz, 2H), 8.49 (br s, 2H), 7.79 (br s, 4H), 4.74 (br s, 4H), 2.08 (br s, 4H), 1.41 (br s, 8 H), 1.34 (br s, 4H); HR-MS (ESI) Anal. Calcd for $C_{32}H_{34}Cl_2N_4$: $[M]^{++}$ 273.1153; found: 273.1150; FTIR (ATR) ν 2987w, 2921m, 2852m, 2759w, 2698w, 2652w, 1650s, 1570m, 1519m, 1490s, 1453s, 1401w, 1349w, 1326m, 1278s, 1249m, 1171w, 1155w, 1130m, 1068s, 873s, 826w, 812s, 765m, 730s, 685m, 630s; RP-HPLC: t_R = 18.77 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 1 min).

4.2.10. Dodecan-1,12-diyl homo-dimer (22)

1,12-Dibromo-dodecane (41 mg, 0.125 mmol, 1.00 equiv) was transformed according to *GP 1*. Recrystallization from *i*PrOH/MeOH gave **22** (65 mg, 0.089 mmol, 71%). Light yellow powder. Mp >200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.31 (br s, 2H), 8.71 (d, J = 6.5 Hz, 2H), 8.60 (d, J = 6.5 Hz, 2H), 8.50 (br s, 2H), 7.79 (m, 4H), 4.74 (t, J = 7.5 Hz, 4H), 2.08 (m, 4H), 1.41 (br s, 4H), 1.34 (br s, 12H); HR-MS (ESI) Anal. calcd for $C_{34}H_{38}Cl_2N_4$: [M]++ 287.1310; found: 287.1312; FTIR (ATR) ν 3398br, 3053w, 3017w, 2923s, 2852s, 2764w, 2699w, 2651w, 1649s, 1573m, 1519m, 1491s, 1455s, 1350w, 1322m, 1280s, 1252m, 1221w, 1171w, 1153m, 1130w, 1069s, 1022w, 873s, 816s, 762w, 727m, 692w, 630s; RP-HPLC: t_R = 20.05 min (C_{18}, 5–50% A in 17 min; 50% A for 3 min, 50–5% A in 11 min).

4.2.11. 6-Bromo-2-methyl-9*H*-beta-carbolin-2-ium iodide (23)

To a solution of 6-bromo-norharmane (**2**) (500 mg, 2.47 mmol, 1.00 equiv) in CH_3CN (15 mL) was added methyl iodide (380 μL, 6.18 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 18 h. The reaction was cooled with an ice-bath, the precipitate filtered, washed with CH_3CN and Et_2O. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **23** (685 mg, 1.76 mmol, 71%) as a yellow solid. An analytical sample was recrystallized (MeOH) for X-ray analysis. Mp = 292.0–293.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.28 (s, 1H), 8.71 (d, J = 6.4 Hz, 1H), 8.67 (d, J = 2.0 Hz, 1H), 8.57 (d, J = 6.4 Hz, 1H), 7.94 (dd, J_1 = 8.7 Hz, J_2 = 1.6 Hz, 1H), 7.75 (d, J = 9.1 Hz, 1H), 4.58 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.1, 135.8, 134.8, 133.3, 131.9, 130.4, 125.6, 121.1, 117.9, 114.4, 114.4, 48.5; HRMS-ESI calcd for $C_{12}H_{10}N_2Br$: $[M]^+$ 261.0027; found 261.0029; FTIR ν 3040m, 1643m, 1566w, 1520w, 1485s, 1447s, 1323m, 1277s, 1254s, 1146m, 1123m, 1053m, 872m, 810s, 729m, 694m cm^{-1}.

4.2.12. Crystallographic details for compound 23

A total of 2517 reflections ($-13 \leqslant h \leqslant 13$, $-19 \leqslant k \leqslant 19$, $-6 \leqslant l \leqslant 9$) were collected at T = 140(2) K in the range of 3.03–26.37° of which 2517 were unique; Mo Kα radiation (λ = 0.71073 Å). The residual peak and hole electron densities were 4.346 and −1.602 eÅ^{-3}, respectively. The absorption coefficient was 5.772 mm^{-1}. The least squares refinement converged normally with residuals of R(F) = 0.0561 (observed data), $wR(F^2)$ = 0.1530 (all data) and a GoF = 1.129. $C_{12}H_{10}BrIN_2$, M_w = 389.03, space group $P2_1/c$, monoclinic, a = 10.7180(9), b = 15.5515(16), c = 7.4595(6) Å, β = 93.188(8)°, V = 1241.42(19) Å^3, Z = 4, ρ_{calcd} = 2.081 Mg/m^3. CCDC-755505 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.2.13. 6-Bromo-2-ethyl-9*H*-beta-carbolin-2-ium iodide (24)

To a solution of 6-bromo-norharmane (**2**) (15.0 mg, 0.06 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added ethyl iodide (12 μL, 0.15 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 15 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **24** (20.0 mg, 0.05 mmol, 83%) as a crystalline solid. Mp = 226.5–227.5 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.37 (s, 1H), 8.74 (d, J = 6.4 Hz, 1H), 8.68 (d, J = 1.6 Hz, 1H), 8.65 (dd, J_1 = 6.4 Hz, J_2 = 1.2 Hz, 1H), 7.94 (dd, J_1 = 8.7 Hz, J_2 = 2.0 Hz, 1H), 7.76 (d, J = 8.7 Hz, 1H), 4.84 (q, J = 7.2 Hz, 2H), 1.76 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.3, 136.0, 134.8, 132.2, 132.1, 129.3, 125.6, 121.2, 118.2, 114.4, 114.4, 56.9, 16.0; HRMS-ESI calcd for $C_{13}H_{12}N_2Br$: $[M]^+$ 275.0184; found 275.0192; FTIR ν 3514w, 3055s, 2955m, 1647s, 1612w, 1516m, 1493s, 1450s, 1319m, 1281s, 1250s, 1165m, 1142s, 1053s, 937m, 868s, 814s, 802s, 725s cm^{-1}.

4.2.14. 2-Allyl-6-bromo-9*H*-beta-carbolin-2-ium bromide (25)

To a solution of 6-bromo-norharmane (**2**) (15.0 mg, 0.06 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added allyl bromide (13 μL, 0.15 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 15 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **25** (14.6 mg, 0.04 mmol, 66%) as a crystalline solid. An analytical sample was recrystallized (Et_2O/hexane) for X-ray analysis. Mp = 195.0–196.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.35 (s, 1H), 8.75 (d, J = 6.4 Hz, 1H), 8.68 (d, J = 1.6 Hz, 1H), 8.62 (dd, J_1 = 6.4 Hz, J_2 = 1.2 Hz, 1H), 7.95 (dd, J_1 = 8.7 Hz, J_2 = 2.0 Hz, 1H), 7.76 (d, J = 8.7 Hz, 1H), 6.30 (m, 1H), 5.57 (dd, J_1 = 9.9, J_2 = 1.2 Hz), 5.56 (dd, J_1 = 15.9 Hz, J_2 = 1.2 Hz), 5.43 (d, J = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.3, 135.9, 135.0, 132.5, 132.4, 131.4, 129.6, 125.7, 121.3, 121.1, 118.2, 114.5, 114.4, 63.0; HRMS-ESI calcd for $C_{14}H_{12}N_2Br$: $[M]^+$ 287.0184; found 287.0179; FTIR ν 3024m, 2997m, 1643s, 1570w, 1512m, 1489s, 1450s, 1358w, 1315m, 1281s, 1254s, 1123s, 1053s, 991m, 937s, 833s, 814s, 768s, 725m cm^{-1}.

4.2.15. Crystallographic details for compound 25

A total of 51084 reflections ($-10 \leqslant h \leqslant 10$, $-29 \leqslant k \leqslant 29$, $-9 \leqslant l \leqslant 9$) were collected at T = 100(2) K in the range of 3.37–27.50° of which 3031 were unique (R_{int} = 0.0461); Mo Kα radiation (λ = 0.71073 Å). The residual peak and hole electron densities were 0.604 and −0.660 eÅ^{-3}, respectively. The absorption coefficient was 6.123 mm^{-1}. The least squares refinement converged normally with residuals of R(F) = 0.0260 (observed data), $wR(F^2)$ = 0.0625 (all data) and a GoF = 1.153. $C_{14}H_{12}Br_2N_2$, M_w = 368.08, space group $P2_1/c$, monoclinic, a = 8.1536(7), b = 22.969(2), c = 7.4050(6) Å, β = 107.832(8)°, V = 1320.2(2) Å^3, Z = 4, ρ_{calcd} = 1.852 Mg/m^3. CCDC-755506 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.2.16. 2-Butyl-6-bromo-9*H*-beta-carbolin-2-ium iodide (26)

To a solution of 6-bromo-norharmane (**2**) (15.0 mg, 0.06 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added iodobutane (17 μL, 0.15 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 15 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **26** (11.0 mg, 0.026 mmol, 43%) as a crystalline solid. Mp = 231.5–232.5 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.38 (s, 1H), 8.73 (d, J = 6.4 Hz, 1H), 8.66 (d, J = 1.2 Hz, 1H), 8.65 (dd, J_1 = 6.4 Hz, J_2 = 1.2 Hz, 1H), 7.93 (dd, J_1 = 9.1 Hz, J_2 = 2.0 Hz, 1H), 7.75 (d, J = 8.3 Hz, 1H), 4.80 (t, J = 7.5 Hz, 2H), 2.12 (quint, J = 7.5 Hz, 2H), 1.50 (sext, J = 7.5 Hz, 2H), 1.06 (t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.2, 135.9, 134.9, 132.5, 132.2, 129.5, 125.6, 121.1, 118.1, 114.4, 114.4, 61.3, 33.6, 19.2, 12.5; HRMS-ESI calcd for $C_{15}H_{16}N_2Br$: $[M]^+$ 303.0497; found 303.0508; FTIR ν 3028s, 2994s, 2955s, 2855m, 1647m, 1570w, 1516m, 1489s, 1447s, 1319m, 1281s, 1254m, 1165m, 1138s, 1049s, 1022w, 903w, 864s, 802s, 725s cm^{-1}.

4.2.17. 6-Bromo-2-(4-methoxycarbonyl-butyl)-9*H*-beta-carbolin-2-ium bromide (27)

To a solution of 6-bromo-norharmane (**2**) (15.0 mg, 0.06 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added methyl bromovalerate (22 μL, 0.15 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 15 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **27** (20.9 mg, 0.047 mmol, 79%) as a crystalline solid. Mp = 203.5–204.5 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.39 (s, 1H), 8.66–8.65 (m, 2H), 8.66 (dd, J_1 = 6.8 Hz, J_2 = 1.2 Hz, 1H), 7.94 (dd, J_1 = 8.7 Hz, J_2 = 1.6 Hz, 1H), 7.75 (d, J = 8.7 Hz, 1H), 4.82 (t, J = 7.5 Hz, 2H), 3.68 (s, 3H), 2.48 (t, J = 7.2 Hz, 2H), 2.17 (quint, J = 7.5 Hz, 2H), 1.74 (quint, J = 7.2 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.8, 143.2, 135.9, 134.9, 132.5, 132.2, 129.6, 125.7, 121.1, 118.1, 114.4, 114.4, 61.0, 50.8, 32.4, 30.8, 21.1; HRMS-ESI calcd for $C_{17}H_{18}N_2O_2Br$: $[M]^+$ 361.0552; found 361.0555; FTIR ν 3024s, 2986s, 2947s, 2913s, 2843m, 1736s, 1643s, 1612w, 1574w, 1520m, 1489s, 1447s, 1366m, 1285s, 1242s, 1200s, 1153s, 1126s, 1092m, 972m, 922m, 872s, 826s, 741s cm^{-1}.

4.2.18. 2-Benzyl-6-bromo-9H-beta-carbolin-2-ium bromide (28)

To a solution of 6-bromo-norharmane (**2**) (15.0 mg, 0.06 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added benzyl bromide (18 μL, 0.15 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 15 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **28** (25.0 mg, 0.06 mmol, quant.) as a crystalline solid. Mp = 235.5–236.5 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.45 (s, 1H), 8.73 (d, J = 6.0 Hz, 1H), 8.71 (dd, J_1 = 6.4 Hz, J_2 = 1.2 Hz, 1H), 8.66 (dd, J_1 = 2.0 Hz, J_2 = 0.8 Hz, 1H), 7.93 (dd, J_1 = 8.7 Hz, J_2 = 2.0 Hz, 1H), 7.74 (d, J = 8.3 Hz, 1H), 7.57 (dd, J_1 = 7.9 Hz, J_2 = 2.0 Hz, 2H), 7.51–7.47 (m, 3H), 5.99 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.3, 135.9, 135.0, 134.3, 132.5, 132.4, 129.5, 129.4, 129.2, 128.4, 125.7, 121.1, 118.3, 114.5, 114.4, 64.1; HRMS-ESI calcd for $C_{18}H_{14}N_2Br$: $[M]^+$ 337.0340; found 337.0336; FTIR ν 3021m, 2986m, 2943m, 2889m, 2843m, 1647m, 1566w, 1520w, 1489s, 1454s, 1319m, 1281s, 1254m, 1200w, 1161m, 1126m, 1053m, 1026w, 868m, 814s, 733s, 706s cm^{-1}.

4.2.19. 6-Bromo-2-(4-fluoro-benzyl)-9*H*-beta-carbolin-2-ium bromide (29)

To a solution of 6-bromo-norharmane (**2**) (25.0 mg, 0.10 mmol, 1.00 equiv) in CH_3CN (1.5 mL) was added 4-fluorobenzylbromide (31 μL, 0.25 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 1 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **29** (42.2 mg, 0.096 mmol, 96%) as a crystalline solid. Mp = 279.5–280.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.43 (s, 1H), 8.73–8.66 (m, 3H), 7.94 (dd, J_1 = 8.8 Hz, J_2 = 1.7 Hz, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.62 (dd, J_1 = 8.5 Hz, J_2 = 5.4 Hz, 2H), 7.23 (t, J = 8.5 Hz, 2H), 5.97 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 163.8 (d, J = 248.0 Hz), 143.7, 136.3, 135.5, 132.9, 132.8, 131.2 (d, J = 8.8 Hz), 130.8 (d, J = 3.2 Hz), 129.9, 126.1, 121.5, 118.7, 116.4 (d, J = 22.1 Hz), 114.9, 114.8, 63.6; HRMS-ESI calcd for $C_{18}H_{13}FN_2Br$: $[M]^+$ 355.0246; found 355.0232; FTIR ν 3040m, 2982m, 2943m, 2886m, 1647m, 1605w, 1508s, 1489s, 1454s, 1350m, 1277s, 1250m, 1223s, 1165s, 1119s, 1053m, 826s, 779s, 698s cm^{-1}.

4.2.20. 6-Bromo-2-(3-fluoro-benzyl)-9*H*-beta-carbolin-2-ium bromide (30)

To a solution of 6-bromo-norharmane (**2**) (25.0 mg, 0.10 mmol, 1.00 equiv) in CH_3CN (1.5 mL) was added 3-fluorobenzylbromide (31 μL, 0.25 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 22 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was

concentrated and dried under high vacuum affording **30** (19.3 mg, 0.044 mmol, 44%) as a crystalline solid. Mp = 237.0–238.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.48 (s, 1H), 8.74 (d, J = 6.8 Hz, 1H), 8.72 (dd, J_1 = 6.4 Hz, J_2 = 1.2 Hz, 1H), 8.64 (d, J = 2.0 Hz, 1H), 7.91 (dd, J_1 = 9.1 Hz, J_2 = 2.0 Hz, 1H), 7.74 (d, J = 9.1 Hz, 1H), 7.54–7.50 (m, 1H), 7.40–7.36 (m, 2H), 7.22 (ddd, J_1 = 9.1 Hz, J_2 = 8.3 Hz, J_3 = 2.4 Hz, 1H), 6.02 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.4 (d, J = 247.4 Hz), 145.6, 138.9 (d, J = 8.2 Hz), 138.1, 137.3, 134.8, 134.7, 133.4 (d, J = 8.2 Hz), 131.9, 128.0, 126.5 (d, J = 2.7 Hz), 123.3, 120.6, 118.4 (d, J = 21.1 Hz), 117.6 (d, J = 23.9 Hz), 116.8, 116.7, 65.5; HRMS-ESI calcd for $C_{18}H_{13}FN_2Br$: $[M]^+$ 355.0246; found 355.0232; FTIR ν 3453w, 3040m, 2947m, 2893m, 2839m, 2696w, 1643m, 1593m, 1516m, 1485s, 1450s, 1319m, 1281s, 1254s, 1150m, 1123s, 1053m, 876m, 806s, 752s cm^{-1}.

4.2.21. 6-Bromo-2-(4-nitro-benzyl)-9*H*-beta-carbolin-2-ium bromide (31)

To a solution of 6-bromo-norharmane (**2**) (15.0 mg, 0.06 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added 4-nitrobenzyl bromide (32.4 mg, 0.15 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 5 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **31** (27.6 mg, 0.060 mmol, 99%) as a crystalline solid. Mp = 261.0–262.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.52 (s, 1H), 8.79 (d, J = 6.4 Hz, 1H), 8.74 (d, J = 6.0 Hz, 1H), 8.70 (s, 1H), 8.34 (d, J = 8.3 Hz, 2H), 7.96 (d, J = 8.3 Hz, 1H), 7.78–7.74 (m, 3 H), 6.16 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 148.5, 143.5, 141.2, 136.0, 135.3, 132.8, 132.7, 130.1, 129.3, 125.8, 124.0, 121.1, 118.5, 114.7, 114.5, 62.8; HRMS-ESI calcd for $C_{18}H_{13}N_3O_2Br$: $[M]^+$ 382.0191; found 382.0183; FTIR ν 3140w, 3048m, 3009m, 2955w, 2855w, 1643m, 1605w, 1516s, 1489s, 1450m, 1339s, 1285s, 1258m, 1223m, 1161m, 1057w, 945m, 856m, 818s, 729s cm^{-1}.

4.2.22. 6-Bromo-2-(3-phenyl-propyl)-9*H*-beta-carbolin-2-ium bromide (32)

To a solution of 6-bromo-norharmane (**2**) (15.0 mg, 0.06 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added 1-bromo-3-phenylpropane (23 μL, 0.15 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 15 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **32** (25.2 mg, 0.056 mmol, 94%) as a crystalline solid. Mp = 257.5–258.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.30 (s, 1H), 8.68 (d, J = 6.8 Hz, 1H), 8.65 (d, J = 1.6 Hz, 1H), 8.62 (dd, J_1 = 6.4 Hz, J_2 = 0.8 Hz, 1H), 7.93 (dd, J_1 = 9.1 Hz, J_2 = 2.0 Hz, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.25 (s, 2H), 7.24 (s, 2H), 7.12 (m, 1H), 4.83 (t, J = 7.5 Hz, 2H), 2.83 (t, J = 7.2 Hz, 2H), 2.49 (quint, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.1, 140.0, 135.8, 134.9, 132.4, 132.2, 129.6, 128.2, 128.0, 125.9, 125.6, 121.1, 118.1, 114.4, 114.3, 61.2, 32.6, 32.1; HRMS-ESI calcd for $C_{20}H_{18}N_2Br$: $[M]^+$ 365.0653; found 365.0653; FTIR ν 3410w, 3024s, 2986s, 2943s, 2839m, 1639s, 1609m, 1570w, 1516w, 1489s, 1450s, 1315m, 1281s, 1254s, 1157s, 1126s, 1049m, 972w, 907w, 876s, 826s, 822s, 733s, 694s cm^{-1}.

4.2.23. 6-Bromo-2-naphthalen-2-ylmethyl-9*H*-beta-carbolin-2-ium bromide (33)

To a solution of 6-bromo-norharmane (**2**) (15.0 mg, 0.06 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added 2-bromomethyl naphthalene (33.2 mg, 0.15 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C for 5 h. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **33** (22.9 mg, 0.049 mmol, 82%) as a crystalline solid. Mp = 223.5–224.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.49 (s, 1H), 8.78–8.73 (m, 2H), 8.67 (d, J = 1.6 Hz, 1H), 8.11 (s, 1H), 7.99–7.91 (m, 4H), 7.75 (d, J = 9.1 Hz, 1H), 7.60–7.58 (m, 3H), 6.15 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.3, 136.0, 135.1, 133.6, 133.4, 132.6, 132.5, 131.5, 129.6, 129.3, 128.3, 127.9, 127.5, 127.0, 126.7, 125.7, 124.9, 121.1, 118.3, 114.5, 114.4, 64.3; HRMS-ESI calcd for $C_{22}H_{16}N_2Br$: $[M]^+$ 387.0497; found 387.0499; FTIR ν 3615w, 3537w, 3368w, 3040m, 2986m, 2936m, 2882m, 2839m, 2797m, 2646w, 1643m, 1609w, 1520m, 1489s, 1450m, 1319m, 1281s, 1157m, 1126s, 1053m, 968w, 872m, 818s, 775s, 733s, 706m cm^{-1}.

4.2.24. 8-Bromo-2-ethyl-9*H*-beta-carbolin-2-ium iodide (35)

To a solution of 8-bromo-norharmane (**34**) (10.0 mg, 0.05 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added ethyl iodide (10 μL, 0.13 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **35** (4.60 mg, 0.011 mmol, 23%) as a crystalline solid. Mp = 292.0–293.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.28 (s, 1H), 8.77 (d, J = 6.4 Hz, 1H), 8.70 (d, J = 6.4 Hz, 1H), 8.48 (d, J = 7.9 Hz, 1H), 8.06 (d, J = 7.5 Hz, 1H), 7.45 (t, J = 7.9 Hz, 1H), 4.86 (q, J = 7.9 Hz, 2H), 1.77 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.1, 135.9, 134.4, 133.7, 132.6, 129.3, 123.0, 122.4, 121.1, 118.5, 105.1, 57.0, 16.0; HRMS-ESI calcd for $C_{13}H_{12}N_2Br$: $[M]^+$ 275.0184; found 275.0182; FTIR ν 3356w, 3048m, 3009m, 2326w, 1643m, 1555m, 1497m, 1470s, 1327s, 1246m, 1215m, 1130s, 1034m, 837s, 791s, 748s cm^{-1}.

4.2.25. 2-Allyl-8-bromo-9*H*-beta-carbolin-2-ium bromide (36)

To a solution of 8-bromo-norharmane (**34**) (10.0 mg, 0.05 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added allyl bromide (11 μL, 0.13 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **36** (4.50 mg, 0.012 mmol, 24%) as a crystalline solid. Mp = 220.0–221.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.25 (s, 1H), 8.79 (d, J = 6.4 Hz, 1H), 8.66 (dd, J_1 = 6.4 Hz, J_2 = 1.2 Hz, 1H), 8.49 (dd, J_1 = 7.9 Hz, J_2 = 0.8 Hz, 1H), 8.07 (dd, J_1 = 7.5 Hz, J_2 = 0.8 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 6.34–6.26 (m, 1H), 5.58 (dd, J_1 = 10.3 Hz, J_2 = 1.2 Hz, 1H), 5.57 (dd, J_1 = 16.7 Hz, J_2 = 1.2 Hz, 1H), 5.45 (d, J = 6.4 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.2, 135.8, 134.5, 134.0, 133.0, 131.4, 129.5, 123.1, 122.4, 121.4, 121.0, 118.5, 105.1, 63.1; HRMS-ESI calcd for $C_{14}H_{12}N_2Br$: $[M]^+$ 287.0184; found 287.0178; FTIR ν 3352w, 3051m, 3017m, 2974m, 2905m, 2858m, 1647m, 1616w, 1558m, 1501m, 1470s, 1327s, 1300m, 1219m, 1138m, 1115m, 1034m, 1011m, 953m, 810m, 783s, 745s, 683m cm^{-1}.

4.2.26. 2-Benzyl-8-bromo-9*H*-beta-carbolin-2-ium bromide (37)

To a solution of 8-bromo-norharmane (**34**) (10.0 mg, 0.05 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added benzyl bromide (15 μL, 0.13 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **37** (9.10 mg, 0.022 mmol, 44%) as a crystalline solid. Mp = 235.0–236.0 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 9.34 (s, 1H), 8.77 (d, J = 6.4 Hz, 1H), 8.75 (d, J = 6.4 Hz, 1H), 8.46 (d, J = 7.9 Hz, 1H), 8.04 (d, J = 7.5 Hz, 1H), 7.58–7.56 (m, 2H), 7.53–7.48 (m, 3H), 7.43 (t, J = 7.5 Hz, 1H), 6.02 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ

143.2, 135.8, 134.5, 134.2, 133.9, 133.1, 129.5, 129.4, 129.3, 128.5, 123.1, 122.4, 121.0, 118.6, 105.1, 64.1; HRMS-ESI calcd for $C_{18}H_{14}N_2Br$: $[M]^+$ 337.0340; found 337.0350; FTIR ν 3399w, 3055m, 3017m, 1643m, 1562w, 1520m, 1497m, 1470s, 1454m, 1327s, 1254m, 1119m, 1034w, 818m, 787m, 748s, 706s cm^{-1}.

4.2.27. 8-Bromo-2-(4-fluoro-benzyl)-9*H*-beta-carbolin-2-ium bromide (38)

To a solution of 8-bromo-norharmane (**34**) (10.0 mg, 0.05 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added 4-fluorobenzyl bromide (16 μL, 0.13 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **38** (5.50 mg, 0.013 mmol, 25%) as a crystalline solid. Mp = 259.5–260.5 °C; ^1H NMR (500 MHz, CD_3OD) δ 9.34 (s, 1H), 8.78 (d, J = 6.8 Hz, 1H), 8.74 (dd, J_1 = 6.4 Hz, J_2 = 0.8 Hz, 1H), 8.47 (d, J = 8.3 Hz, 1H), 8.05 (d, J = 7.9 Hz, 1H), 7.64 (dd, J_1 = 8.7 Hz, J_2 = 5.2 Hz, 2H), 7.44 (t, J = 7.9 Hz, 1H), 7.25 (t, J = 8.7 Hz, 2H), 6.01 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 163.5 (d, J = 248.4 Hz), 143.2, 135.8, 134.6, 134.0, 133.0, 130.9 (d, J = 8.2 Hz), 130.23 (d, J = 2.7 Hz), 129.4, 123.1, 122.5, 121.0, 118.7, 116.1 (d, J = 22.9 Hz), 105.1, 63.2; HRMS-ESI calcd for $C_{18}H_{13}N_2BrF$: $[M]^+$ 355.0246; found 355.0257; FTIR ν 3364w, 3044m, 3001m, 2978m, 1647m, 1562m, 1512m, 1497m, 1470s, 1327s, 1250m, 1138m, 1115m, 860m, 810m, 783s, 748s cm^{-1}.

4.2.28. 8-Bromo-2-naphthalen-2-ylmethyl-9*H*-beta-carbolin-2-ium bromide (39)

To a solution of 8-bromo-norharmane (**34**) (10.0 mg, 0.05 mmol, 1.00 equiv) in CH_3CN (0.5 mL) was added 2-bromomethyl naphthalene (28 mg, 0.13 mmol, 2.50 equiv). The flask was sealed and heated at 85 °C overnight. The reaction was cooled to rt, the precipitate filtered, washed with CH_3CN and pentane. The product was dissolved in MeOH and any precipitate removed by filtration. The filtrated was concentrated and dried under high vacuum affording **39** (10.0 mg, 0.021 mmol, 43%) as a crystalline solid. Mp = 244.5–245.0 °C; ^1H NMR (500 MHz, CD_3OD) δ 9.40 (s, 1H), 8.81 (dd, J_1 = 6.4 Hz, J_2 = 1.2 Hz, 1H), 8.77 (d, J = 6.4 Hz, 1H), 8.46 (d, J = 7.9 Hz, 1H), 8.12 (s, 1H), 8.03 (d, J = 7.5 Hz, 1H), 7.99 (d, J = 8.7 Hz, 1H), 7.97 (dd, J_1 = 6.0 Hz, J_2 = 3.6 Hz, 1H), 7.93 (dd, J_1 = 6.0 Hz, J_2 = 3.6 Hz, 1H), 7.61–7.58 (m, 3H), 7.43 (t, J = 7.9 Hz, 1H), 6.19 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 143.2, 135.8, 134.5, 134.0, 133.7, 133.4, 133.2, 131.4, 129.5, 129.3, 128.4, 127.9, 127.5, 127.0, 126.8, 125.0, 123.1, 122.4, 121.0, 118.6, 105.1, 64.3; HRMS-ESI calcd for $C_{22}H_{16}N_2Br$: $[M]^+$ 387.0497; found 387.0512; FTIR ν 3372m, 3051m, 3013m, 2928m, 1643m, 1562w, 1516m, 1474m, 1331s, 1258m, 1126s, 1034w, 864m, 806s, 783s, 752s cm^{-1}.

4.3. Bacteria and MIC determination

Actinobacterial species used in this study were *C. glutamicum* ATCC13032, *M. smegmatis* mc^2155 and *M. tuberculosis* H37Rv. These were grown in 7H9 medium and tested for susceptibility to nostocarboline derivatives using the resazurin-reduction method.[33] The minimal inhibitory concentration (MIC_{99}) was defined as the lowest drug concentration that prevented growth of 99% of the cells.

4.4. Determination of antiprotozoal and cytotoxic activity

In vitro assays with *T. brucei rhodesiense* STIB 900 bloodstream forms, *P. falciparum* K1 erythocytic stages, *T. cruzi* Tulahuen C2C4 amastigotes in L6 cells (rat skeletal myoblasts) and *L. donovani* MHOM-ET/67/L82 axenic amastigotes as well as for cytotoxicity using L6 cells were carried out as previously reported.[34]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.013.

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